SEQUENCE LISTING

A sequence listing was submitted with the response to missing parts filed on September 19, 2001. A copy of the Sequence Listing with diskette is enclosed.

INFORMATION DISCLOSURE STATEMENT

Pages 2-4 of the 1449 submitted on August 16, 2001 were initialed and returned by the Examiner with the office action. Page 1 of the 1449 form was missing and is therefore resubmitted for consideration by the Examiner.

COMMENTS ON THE SPECIFICATION

The specification was objected to for various sequence listing informalities that are corrected with this amendment. The description of the figures was also objected to for use of the phraseology "panel" in reference to panels A, B, C, etc. for given figures. The relevant passages in the brief description of the figures have been amended to remove the word "panel," and to address the other formal issues noted by the Examiner. Accordingly, the objections should be withdrawn.

DOUBLE PATENTING

Claims 1, 5-11, 13-17, 22, 23, 25-27 and 36-38 were rejected for alleged obviousness-type double patenting over commonly owned patent 6,436,646 in view of 5,800,989. Without taking any position on the rejection, Applicants note that they are willing to submit a terminal disclaimer to overcome the rejection, if such a disclaimer is appropriate when the relevant claims are indicated to be otherwise allowable. A terminal disclaimer will overcome the rejection, as helpfully noted by the Examiner.

ALL OBVIOUSNESS REJECTIONS RELYING ON 6,287,774 AND 6,436,646

Various claims were prospectively rejected under 103(a) over 6,287,774 and/or 6,436,646 in combination with additional references. The Examiner helpfully notes that these rejections fall away upon an indication by Applicants that the '774 and '646 patents were commonly owned with the subject case when the invention was made. Applicants hereby

confirm that all of the cases shared common ownership when the invention was made. Further, Applicants note that the applications continue to share common ownership. Evidence of common ownership is submitted in the form of the recorded assignment documents for each of the respective applications/ patents (Exhibit A). Accordingly, the provisions of 35 USC § 103(c) apply and the rejection must be withdrawn. *Applicants note that claim 2 was rejected solely for alleged obviousness over such commonly owned patents and is, therefore, clearly allowable as written.*

35 U.S.C. §112, SECOND PARAGRAPH.

Claims 3 and 4 were rejected under 35 U.S.C. §112, second paragraph, due to alleged uncertainty as to the scope of the term "polyion." Applicants have changed the term to "polymeric polyion" as helpfully suggested by the Examiner. Accordingly, the rejection should be withdrawn.

Claims 22 and 23 were rejected for alleged indefiniteness due to perceived confusion with respect to claim 16. Applicants respectfully traverse this rejection, noting that claim 16 refers to the time at which hybridization occurs, while claims 22 and 23 refer to other operations (detection of FP, determination of FP over time). Accordingly, there is no logical conflict between the language of the claims. This rejection should be withdrawn.

LINN DOES NOT ANTICIPATE THE CLAIMED INVENTION

Claims 1, 3-7, 9, 11, 14-17, 22, 23, 25 and 34-37 were rejected for alleged anticipation over Linn, USP 5,800,989. Applicants respectfully traverse.

The rejection alleges that the claims are anticipated because Linn mentions the possibility of using rhodamine and Texas Red dyes to label nucleic acids for use in the Linn methods, citing column 9, line 49.

As an initial matter, Applicants note that the rest of the Linn patent describes the use of fluorescein labels, rather than rhodamine or Texas Red. The specification extols the supposed benefits of fluorescein throughout column 10. There is no indication that the method was ever actually practiced by Linn using rhodamine or Texas Red labels. Thus, the surprising discovery of Applicants that neutral or positively charged labels provide a dramatic improvement

in FP signal is not ever mentioned by Linn, nor does Linn appear to have been aware of it. Indeed, the supposed point of the Linn reference is that DNA binding proteins are used to enhance FP for DNA duplexes—an option that does not rely on the advantages of a positive or neutral dye in the duplex.

Accordingly, the issue is whether the simple listing of Rhodamine and Texas Red in Linn is equivalent to a teaching that positively charged or neutral dyes are to be used to enhance FP, as in Applicants' claimed invention (e.g., Claim 1). Applicants respectfully submit that it is not.

That is, the charge nature of the relevant dye is dependent on a variety of factors, including which salt form of the dye is being used, what the pH of the relevant system is, what salt ions are present, whether charge altering groups are included, etc. Essentially any dye can be made positive, neutral or negative by using the appropriate salt form, use of charged linkers, and the like. This is outlined in some detail in the subject application at page 17, line 20- page 19 line 9. This is as true of Texas Red and Rhodamine as it is for any other dye. For example, while certain commonly used sulfonyl chloride salts of Texas Red have a neutral charge, other commonly used forms, such as ammonium salts of Texas Red, are negatively charged. Thus, the simple indication that Texas Red or Rhodamine can be used in a procedure simply does not, by itself, provide any indication of the charge of the dye to be used. Linn never indicates any particular charge state for the dyes to be used in the various FP procedures, which means that the patent flatly fails to teach the elements of the claims at issue.

It is worth noting that Applicants' dependent claims, which include the possibility of, e.g., the use of rhodamine, in no way contradict the foregoing. That is, the limitations of the independent claim are read into the relevant dependent claim, meaning that the claims specify that the form of Rhodamine or Texas Red that is at issue is neutrally or positively charged.

In the complete absence of any teaching of the actual claim limitations at issue, the rejection is improper and should be withdrawn.

LINN AND HYLDIG NIELSEN ARE NOT COMBINABLE.

Claims 8, 10 and 38 were rejected under 35 U.S.C. §103(a) as allegedly obvious over Linn (USP 5,641,633) in light of Hyldig Nielsen (USP 6,280,946). Applicants traverse.

The Action helpfully indicates that Linn does not teach the use of PNAs in the methods of the patent, but then alleges that it would have been a routine substitution to use PNAs, e.g., as taught by Hyldig Nielsen in the Linn methods. Applicants respectfully disagree.

Specifically, the Linn methods explicitly rely upon the binding of DNA binding proteins to double stranded DNAs to overcome the stated difficulties with attempting FP analysis of double stranded DNA, particularly at high temperatures. *See*, e.g., the Linn abstract. In fact there is no teaching, in either reference, that such DNA binding proteins can bind to DNA-PNA hybrids. There is no teaching that, even if they do bind, they will have a similar effect (i.e., to hold the duplex together at high temperatures). Accordingly, no specific motivation to combine the references can be drawn from the references or the art as discussed in the action.

Moreover, given that the physical backbone of a PNA is completely different than that of a DNA, there was no reason to expect that a DNA binding protein would bind to or stabilize the relevant DNA-PNA heteroduplex under the relevant conditions. Thus, neither motivation to combine the references, nor any expectation of successfully combining the references, can be drawn out of the cited art.

Applicants also note that Linn does not teach the relevant limitations of the independent claims as supposed (argued in detail above) and that the addition of Hyldig Nielsen in no way provides the missing elements of the relevant claims. Thus, because the references in combination do not teach the limitations of the claims, the rejection must be withdrawn. As indicated in more detail above, the references are also not combinable as supposed, providing an additional basis for overcoming the rejection of claims 8, 10 and 38.

<u>LINN AND SAKI IN COMBINATION DO NOT TEACH THE INVENTION</u>

Claims 12, 13, 18-21, 24 and 26-32 were rejected for alleged obviousness over Linn in view of Saiki et al. (Nature 324: 163-166). Applicants traverse.

The rejection urges that it would have been obvious to practice the Linn methods in combination with standard allele-specific discrimination methods such as those taught by Saiki et al. Applicants respectfully disagree.

Nothing in Linn indicates that the form of FP being used is sufficiently discriminative to itself distinguish binding of nucleic acids that differ by one or a few nucleic

acid residues. Indeed, given that the Linn method relies on the use of DNA binding proteins to increase FP signals, it is utterly unclear even in light of Applicants invention whether or not the effects of protein binding would completely swamp out the effects of nucleotide differences between nucleic acids during FP. It seems rather more likely, if Linn had thought of the application at all, that they would have attempted to perform the discrimination during the amplification phase, rather than during the FP detection phase (a procedure that would not be relevant to the claims at issue). Thus, there is simply no evidence that the proposed combination would work (and therefore, there can be no expectation of success in practicing the proposed combination of references).

Applicants also note that the rejection provides no specific motivation for combining the references that can be drawn out of the cited prior art. The rejection argues that it would have been obvious to combine the references because one would want to detect SNPs in real time and with higher resolution. In fact, as noted above, there has been no showing that the combination would have this effect; in addition, the procedures of Saiki are allegedly a simplified superior method in and of themselves—there is simply no indication that some completely different method should really be used instead. Contrawise, Linn, who had roughly 10 years to digest the teachings of Saiki provides no indication that his method should be used in place of the Saiki method (possibly because, as noted above, he simply would not have even known whether his method could be used for such an application, at least in the way hypothesized by the Action). It is noted that the courts have repeatedly indicated that there must be a specific motivation for combining references that is drawn out of the art itself—a generalized argument that it would have worked if thought of has consistently been held insufficient to establish motivation to combine.

Finally, as noted above, Linn does not teach the relevant limitations of the independent claims as supposed (argued in detail above). The addition of Saiki et al. in no way provides the missing elements of the relevant claims.

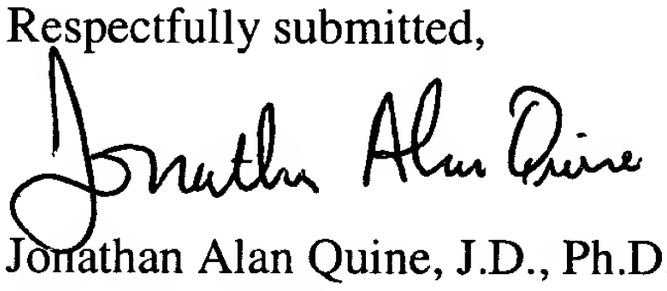
Thus, because there would have been no motivation to combine the references, and because the references in combination do not teach the limitations of the claims, the rejection must be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

Respectfully submitted,



Jonathan Alan Quine, J.D., Ph.D.
Reg. No. 41,261

QUINE INTELLECTUAL PROPERTY LAW GROUP, P.C.
P.O. BOX 458
Alameda, CA 94501
(510) 337-7871
Fax (510) 337-7877

APPENDIX A
MARKED UP VERSION OF AMENDMENT CLAIMS

Page 5, lines 16-19:

Figure 1 is a schematic representation of DNAs and PNAs. The top **[panel]** is a schematic of a single stranded DNA. The middle **[panel]** is a schematic of a single-stranded PNA. The bottom **[panel]** is a schematic overlay of the DNA and PNA, showing the similarity of the overall structure of the two molecules.

Page 5, line 20-28:

Figure 2**[, Panel]**A schematically shows the use of poly-lysine for PNA-DNA hybrid detection. Figure 2**[, Panel]**B shows several histograms summarizing hybridization data for three PNAs. Figure 2**[, Panel]**C shows a histogram depicting the kinetics of PNA/DNA hybridization as detected by FP in the presence of polylysine (*see also*, Anal. Biochem. 275, 248 (1999)). Figure 2**[, Panel]**D shows several melting curves analyzed by FP. Figure 2**[, Panel]**E shows graphs depicting the effects of mismatch position on PNA/DNA duplex stability with PNA probe 188 (a 9-mer). Assay conditions were: 50 nM PNA 188, 50 mM HEPES pH 7.5, 3.3 μM Poly L-Lysine.

Page 5, line 29 – page 6, line 7:

Figure 3**[, Panel]**A depicts a graph showing the effects of mismatch position on PNA/DNA duplex stability for PNA probe 201 (an 11-mer). Assay conditions included: 50 nM PNA 201, 50 mM HEPES pH 7.5, 3.3 μM Poly L-Lysine. Figure 3**[, Panel]**B shows graphs with the results for SNP typing in PCR products, including fluorescein labeled PNA probes. In this experiment, single stranded PCR products were 79 bases long; PNA 7637 is was a 9-mer, matching the pUC product and having a TG mismatch with the pBR product; and PNA 7699 is a 13-mer, fully complementary to both PCR products. Figure 3**[, Panel]**C shows a set of graphs showing SNP discrimination in the absence of polylysine for rhodamine labeled PNAs. Figure 3**[, Panel]**D shows melting curves for rhodamine-labeled probe 8158, including a melting curve for wild-type and G/T SNP targets, in the absence of poly-lysine.

Page 6, lines 8-22:

Figure 4, shows additional histograms and example conclusions for the effect of polylysine on DNA/PNA duplex stability, real time detection of T7 gene 6 exonuclease degradation of a PCR product coupled with PNA probe hybridization and the effect of target size and polylysine. **[Panel]** **Figure 4**A shows a graph of FP vs. temperature including the effect of poly-Lysine on PNA/DNA duplex stability. Experiments were with rhodamine labeled probes at 50mM HEPES pH7.5/ 50mM NaCl, 2μM PNA, 5μM DNA Targets, +/- 4μM pLL. **[Panel]** **Figure 4**B shows histograms for rhodamine labeled PNAs, including the effect of target size and poly-lysine. **[Panel]** **Figure 4**C shows real-time detection of T7 gene 6 exonuclease degradation of a PCR product coupled with PNA probe hybridization. One of the PCR strands contains four phosphorothioates at its 5' end, making it resistant to T7 gene 6 exonuclease. The enzyme hydrolyses the opposite strand to generate a single-stranded template to which the PNA probe hybridizes. The reactions were carried out in PCR buffer. The DNA targets were a 22mer (280) and 9mers (289, 290). PNA probes were 200 nM in 50 mM HEPES pH 7.5, 50 mM NaCl, with Poly-lysine at 0 or 4 μM.

Page 6, lines 25-27:

Figure 7**, Panels A-C,** is a schematic illustration of a microfluidic device incorporating an external sampling pipettor as a reaction/assay receptacle in the present invention.

Page 7, lines 4-5:

Figure**s** 10**A-B** illustrate**[s]** an exemplary computer system and architecture for use with the present invention.

Page 36, lines 26-24:

Sequences of nucleic acids used for the analysis depicted in Figure 2 include: 188: Fl-O-CAA-ATA-CTC; 201: Fl-O-TCA-AAT-ACT-CC **(SEQ ID NO. 1)**; 202: Fl-O-GTC-AAA-TAC-TCC-A **(SEQ ID NO. 2)** (also labeled with BODIPY-Fl); 7637: Fl-O-CCT-GTA-GCA; 7638: Fl-O-TGC-TAC-AGG; 7699: Fl-O-CAC-CAC-GAT-GCC-T **(SEQ ID NO. 3)**; 212 5'

GCTGGAGTATTTGACCT **(SEQ ID NO. 4)**; 244 5' TTGTTGCCAATGCTACAGGCATCGT **(SEQ ID NO. 5)**; 245 5' TTGTTGCCAATGCTGCAGGCATCGT **(SEQ ID NO. 6)**; and 247 5' ACGATGCCTGTAGCATTGGCAACAA **(SEQ ID NO. 7)**. Assay conditions were: 50 nM PNA 188, 50 mM HEPES pH 7.5, 3.3 μM Poly L-Lysine.

Page 39, lines 12-17:

[The disclosure of USSN 60/203,723 is incorporated by reference in its entirety for all purposes. In addition, all publications, patents, patent applications, other documents, internet citations, CD-ROM citations and other publicly accessible information listed herein are hereby incorporated by reference for all purposes, as if each individual publication, patent, patent application or other document was specifically and individually indicated to be incorporated by reference.] **The disclosure of USSN 60/203,723 is incorporated by reference in its entirety for all purposes. In addition, all publications, patents, patent applications, other documents, internet citations, CD-ROM citations and other publicly accessible information listed herein are hereby incorporated by reference for all purposes, as if each individual publication, patent, patent application or other document was specifically and individually indicated to be incorporated by reference.**

APPENDIX B
MARKED UP COPY OF AMENDED CLAIMS

2 (AMENDED). [The method of claim 1] A method for detecting a nucleic acid, the method comprising:

contacting a first nucleic acid to a second nucleic acid, which second nucleic acid comprises a neutral or positively charged fluorescent label; and,detecting fluorescence polarization of the resulting mixture of first and second nucleic acids, wherein the fluorescence polarization is increased by less than about 50% by the addition of polylysine to the first and second nucleic acid.

APPENDIX C
COURTESY COPY OF PENDING CLAIMS

1. A method for detecting a nucleic acid, the method comprising:

contacting a first nucleic acid to a second nucleic acid, which second nucleic acid comprises a neutral or positively charged fluorescent label; and,

detecting fluorescence polarization of the resulting mixture of first and second nucleic acids.

2. (AMENDED). A method for detecting a nucleic acid, the method comprising:

contacting a first nucleic acid to a second nucleic acid, which second nucleic acid comprises a neutral or positively charged fluorescent label; and,detecting fluorescence polarization of the resulting mixture of first and second nucleic acids, wherein the fluorescence polarization is increased by less than about 50% by the addition of polylysine to the first and second nucleic acid.

3. The method of claim 1, wherein the mixture of first and second nucleic acids is present in a composition which is substantially free of polyion.

4. The method of claim 3, wherein the composition comprises less than 1μM polyion.

5. The method of claim 1, wherein a rotational diffusion rate of a duplex of the first and second nucleic acid is less than a rotational diffusion rate of the first or second nucleic acid.

6. The method of claim 5, wherein the fluorescence polarization of unduplexed first or second nucleic acid is at least 50% different than the fluorescence polarization of the duplexed nucleic acid.

7. The method of claim 1, wherein the first or second nucleic acid comprises one or more of: DNA, RNA, LNA, a DNA analogue, an RNA analogue or a PNA.

8. The method of claim 1, wherein one or more of the nucleic acids is nuclease resistant.

9. The method of claim 1, wherein the fluorescent label comprises rhodamine or BODIPY.

10. The method of claim 1, wherein the first nucleic acid is a DNA and the second nucleic acid is a PNA which comprises a rhodamine label.

11. The method of claim 1, wherein the first or second nucleic acids comprise at least a region which is single-stranded.

12. The method of claim 11, wherein the first and second nucleic acid are perfectly complementary.

13. The method of claim 11, wherein the first and second nucleic acid comprise at least one non-complementary nucleotide when aligned for maximum complementarity.

14. The method of claim 11, further comprising determining from the fluorescence polarization detection whether the first and second nucleic acids are duplexed.

15. The method of claim 11, further comprising determining the extent to which the first and second nucleic acids are duplexed from the fluorescence polarization detection.

16. The method of claim 1, wherein the first and second nucleic acids hybridize in solution prior to detection of fluorescence polarization.

17. The method of claim 16, comprising comparing the detected fluorescence polarization to a fluorescence polarization measurement of either the first or the second nucleic acid alone in solution.

18. The method of claim 16, comprising comparing the detected fluorescence polarization to a fluorescence polarization measurement of either the first or the second nucleic acid hybridized to a third nucleic acid.

19. The method of claim 18, wherein the third nucleic acid is perfectly complementary to either the first or the second nucleic acid.

20. The method of claim 18, wherein the third nucleic acid is not perfectly complementary to either the first or the second nucleic acid.

21. The method of claim 18, wherein the third nucleic acid is unrelated in sequence to either the first or the second nucleic acid.

22. The method of claim 16, comprising detecting fluorescence polarization during hybridization of the first and second nucleic acid.

23. The method of claim 22, further comprising determining the fluorescence polarization as a function of time during hybridization of the first and second nucleic acid.

24. The method of claim 23, further comprising plotting a histogram of the fluorescence polarization as a function of time.

25. A method of identifying the presence of a subsequence of nucleotides in a target nucleic acid, the method comprising:

contacting the target nucleic acid sequence with a labeled nucleic acid probe, which labeled nucleic acid probe comprises a neutral or positively charged label comprising a fluorophore to form a first reaction mixture; and,

detecting the level of fluorescence polarization of the first reaction mixture.

26. The method of claim 25, wherein the target nucleic acid sequence comprises at least one locus for a single nucleotide polymorphism.

27. The method of claim 26, wherein the nucleic acid probe is complementary to one allele of the single nucleotide polymorphism in the target nucleic acid sequence.

28. The method of claim 25, comprising contacting a plurality of additional target nucleic acids with a plurality of additional labeled nucleic acid probes, which additional labeled nucleic acid probes individually comprise a neutral or positively charged label comprising a fluorophore to form a plurality of additional reaction mixtures; and,

detecting the level of fluorescence polarization of the plurality of additional reaction mixtures.

29. The method of claim 28, wherein the plurality of additional target nucleic acids individually comprise at least one locus for a single nucleotide polymorphism.

30. The method of claim 29, wherein the plurality of additional nucleic acid probes are individually complementary to at least one allele of each of the single nucleotide polymorphisms in the plurality of target nucleic acid sequences.

31. The method of claim 30, wherein the plurality of additional target nucleic acids are derived from a single species, variety, cultivar, cell, virus, or organism.

32. The method of claim 31, wherein identification of the single nucleotide polymorphisms provides a single nucleotide polymorphism genotype for the species, variety, cultivar, cell, virus or organism.

33. The method of claim 25, wherein the fluorescence polarization is increased by less than about 50% by the addition of polylysine to the target and probe nucleic acids.

34. The method of claim 25, wherein the target and probe nucleic acids are present in a composition which is substantially free of polyion.

35. The method of claim 34, wherein the composition comprises less than 1 μM polyion.

36. The method of claim 25, wherein a rotational diffusion rate of a duplex of the target and probe nucleic acids is less than a rotational diffusion rate of the target or probe nucleic acids.

37. The method of claim 36, wherein the fluorescence polarization of the probe which is duplexed to the target is at least 50% different than the fluorescence polarization of the probe when not duplexed to the target.

38. The method of claim 25, wherein the target or probe nucleic acids comprise one or more of: DNA, RNA, LNA, a DNA analogue, an RNA analogue or a PNA.